Resequencing *Vrs1* gene in Spanish barley landraces revealed reversion of six-rowed to two-rowed spike

Ana M. Casas[1*], Bruno Contreras-Moreira[1,2], Carlos P. Cantalapiedra[1], Shun Sakuma[3,4], María Pilar Gracia[1], Marian Moralejo[5], José Luis Molina-Cano[6], Takao Komatsuda[7], Ernesto Igartua[1]

Concise title: Natural variation in Vrs1

[1]Estación Experimental de Aula Dei, Consejo Superior de Investigaciones Científicas (CSIC), Zaragoza, Spain

[2]Fundación ARAID, Zaragoza, Spain

[3]Faculty of Agriculture, Tottori University, Tottori, Japan

[4]Current address: Leibniz Institute of Plant Genetics and Crop Plant Research, IPK, Gatersleben, Germany

[5]Universitat de Lleida, Lleida, Spain

[6]Institut de Recerca i Tecnología Agroalimentàries, (IRTA), Lleida, Spain (retired)

[7]Institute of Crop Science, National Agriculture and Food Research Organisation, Tsukuba, Japan

*** Correspondence:** Ana M. Casas, acasas@eead.csic.es

Phone: +34 976716085

Abstract

Six-rowed spike 1 (*Vrs1*) is a gene of major importance for barley breeding and germplasm management as is the main gene determining spike row-type (2-rowed vs 6-rowed). This is a widely used DUS trait, and has been often associated to phenotypic traits beyond spike type. Comprehensive re-sequencing *Vrs1* revealed three two-rowed alleles (*Vrs1.b2*; *Vrs1.b3*; *Vrs1.t1*) and four six-rowed (*vrs1.a1*; *vrs1.a2*; *vrs1.a3*; *vrs1.a4*) in the natural population. However, the current knowledge about *Vrs1* alleles and its distribution among Spanish barley subpopulations is still underexploited. We analyzed the gene in a panel of 215 genotypes, made of Spanish landraces and European cultivars. Among 143 six-rowed accessions, 57 had the *vrs1.a1* allele, 83 were *vrs1.a2* and three showed the *vrs1.a3* allele. *Vrs1.b3* was found in most two-rowed accessions, and a new allele was observed in 7 out of 50 two-rowed Spanish landraces. This allele, named *Vrs1.b5*, contains a 'T' insertion in exon 2, originally proposed as the causal mutation giving rise to the six-row *vrs1.a2* allele, but has an additional upstream deletion that results in the change of 15 amino acids and a potentially functional protein. We conclude that eight *Vrs1* alleles (*Vrs1.b2*, *Vrs1.b3*, *Vrs1.b5*, *Vrs1.t1*, *vrs1.a1*, *vrs1.a2*, *vrs1.a3*, *vrs1.a4*) discriminate two and six-rowed barleys. The markers described will be useful for DUS identification, plant breeders, and other crop scientists.

Keywords: barley, landraces, *Vrs1*, SNP

A feature relevant to describe the history of the barley crop *Hordeum vulgare* subsp. *vulgare* is the spike row-type, two- and six-rowed, according to the fertility of the lateral spikelets of each triplet sitting at each rachis node. *H. vulgare* subsp. *spontaneum*, the wild ancestor of barley, is two-rowed, as explained at length in Komatsuda et al. (2007). So far, five genes determining row-type (*vrs1*, *vrs2*, *vrs3*, *vrs4* and *vrs5*) have been cloned. *Vrs1* (syn. *HvHoX1*) encodes a homeodomain-leucine zipper class I (HD-ZIP I) transcription factor that inhibits the development of lateral spikelets (Komatsuda et al. 2007). *Vrs2* encodes a homolog of *SHORT INTERNODES* (Youssef et al. 2017a). *Vrs3* encodes a putative Jumonji histone demethylase (Bull et al. 2017; van Esse et al. 2017). *Vrs4* encodes an orthologue of the maize *RAMOSA 2* gene (Koppolu et al. 2013). The last gene, *Vrs5* (syn. *Int-c*) encodes a homologue of maize *TEOSINTE BRANCHED 1* (Ramsay et al. 2011). However, only two of them, *Vrs1* and *Vrs5*, affect spike row-type in natural populations (Komatsuda et al. 2007; Saisho et al. 2009; Ramsay et al. 2011; Youssef et al. 2017b).

Several possible mutations at *Vrs1* convert the sterile lateral spikelets into fertile ones. These mutations occur naturally and may have been favored by farmers that interpreted increased fertility as a yield-increasing trait, even though biomass productivity differs little between the two forms (Evans and Wardlaw 1976). The six-rowed trait has appeared independently in several occasions during the history of the crop, acting as a driver of germplasm differentiation (von Bothmer et al. 2003; Komatsuda et al. 2007). Actually, the distinction between two-rowed and six-rowed types is one of the main divides in barley germplasm, as breeders tend to maintain their stocks apart, to avoid the cumbersome process of recovery of pure spike types.

In addition to two two-rowed alleles (*Vrs1.b2* and *Vrs1.b3*), at least three independent mutations in *Vrs1* (*vrs1.a1*, *vrs1.a2* and *vrs1.a3*, all six-rowed) are found among current barleys (Komatsuda et al. 2007). These last alleles are caused by a deletion (*vrs1.a1*) or insertion (*vrs1.a2*) in the coding sequence, resulting in frame shifts; whereas *vrs1.a3* is due to an amino acid change in the homeodomain region. Different surveys resequencing the gene across wild and cultivated barleys (Saisho et al. 2009; Cuesta-Marcos et al. 2010; Ramsay et al. 2011; Youssef et al. 2012, 2017b), ancient DNA from historic landraces (Leino and Hagenblad 2010), or through the use of specific KASP markers in herbarium specimens (Lister et al. 2013), also found the previous alleles and identified another two, *Vrs1.t* (*deficiens*), which is caused by a single amino acid change in the C-terminal region of the protein (Sakuma et al. 2017) and *vrs1.a4* (six-rowed, without any apparent change in the open reading frame (ORF) of *Vrs1.b* alleles).

74 The *Vrs1* region is associated not just to row-type. In fact, it turns up in many barley
75 association scans, for a wide variety of agronomic traits (Cuesta-Marcos et al. 2010; Muñoz-
76 Amatriaín et al. 2014; Alqudah et al. 2016). Recently, some studies have provided functional
77 proof of the gene involvement in several phenotypic traits. Liller et al. (2015) found an effect
78 of *Vrs1* on tillering, while Thirolugachandar et al. (2017) reported increased leaf width, vein
79 number, leaf nitrogen content, and grain number associated to the six-rowed allele.

80 Spain is at the end of the routes of distribution that brought barley and other crops to Europe,
81 starting from the Neolithic and ending probably in the Middle Ages (Fischbeck 2003;
82 Komatsuda et al. 2007), receiving crops with adaptations to environmental factors
83 encountered along different routes (Banks et al. 2013; Müller 2015). Therefore, Spanish
84 landraces actually summarize the evolution of the crop in, at least, Southern Europe.
85 Additionally, Spain is one of the few European countries in which cereal landraces were
86 collected and kept in germplasm banks before they disappeared from cultivation, because they
87 were cultivated up to the second half of the 20th century (Igartua et al. 1998; Pujol-Andreu
88 2011).

89 Before cloning *Vrs1*, polymorphisms in the linked *Chloroplast Elongation Factor G* gene
90 (cMWG699), closely linked to the *Vrs1* locus, were used as a surrogate for its row-type
91 characterization (Komatsuda et al. 1998; Tanno et al. 1999, 2002). Cuesta-Marcos et al. (2010)
92 developed four SNP markers within the *Vrs1* gene that have been widely used by the scientific
93 and plant breeding community as diagnostic for row-type. However, the current knowledge
94 about *Vrs1* alleles and its distribution among barley subpopulations is still underexploited. In
95 this work, we carry out a comprehensive survey of haplotypes at this locus by analyzing
96 Spanish barley landraces, which are complementary of barley accessions analyzed in other
97 studies (Saisho et al. 2009; Cuesta-Marcos et al. 2010; Ramsay et al. 2011; Sakuma et al. 2017;
98 Youssef et al. 2017b), with a predominance of six-rowed over two-rowed accessions.

99

100 **Materials and Methods**

101 **Plant materials**

102 This study involved 176 Spanish barley landraces (50 two-rowed and 126 six-rowed), most of
103 them collected before 1954 (Supplementary Table 1). Of these landraces, 137 (126 six-rowed
104 and 11 two-rowed) belonged to the Spanish Barley Core Collection (SBCC, Igartua et al. 1998)

and 39 come from the set assembled by Moralejo et al. (1994). Thirty-six additional cultivars originated from other countries were studied for comparative purposes: 8 landraces from Morocco (4 two-rowed and 4 six-rowed), 7 of them obtained from the USDA World collection, and 28 widely diverse cultivars (15 two-rowed and 13 six-rowed) that represented the cultivated gene pool from the world. Finally, we included 3 wild barley accessions from Morocco (Molina-Cano et al. 1982) donated by J.L. Molina-Cano (Table 1).

SNPs genotyping

Four SNPs within the *Vrs1* gene (HORVU2Hr1G092290 in the new barley genome sequence (Mascher et al. 2017)) were genotyped as a part of the 9k Infinium iSelect SNP chip (Comadran et al. 2012). Two of those SNPs (12_30897 and 12_30901) were included in the Barley SNP Panel from Eureka Genomics Corporation (Hercules, CA). The CAPs marker cMWG699/*Taq*I (Komatsuda et al. 1998; Tanno et al. 1999), corresponding to HORVU2Hr1G092180, was evaluated in all the samples as previously described (Casas et al. 2005). The plant materials tested with each system are detailed in Table 1.

Sanger sequencing

Sanger-sequencing of *Vrs1* and *Int-c* (HORVU4Hr1G007040) of selected accessions were carried out as described by Ramsay et al. (2011).

Exome sequencing

Exome capture was performed according to the methods described by Mascher et al. (2013). DNA sequencing, made at CNAG (Centro Nacional de Análisis Genómico, Barcelona), and data analysis were performed as described in Cantalapiedra et al. (2016). Briefly, mapping of paired-end reads (2x101 bp) to the Morex WGS assembly was carried out with BWA MEM (Li and Durbin 2009). Variant calling was done by combining SAMtools (Li et al. 2009) and GATK (McKenna et al. 2010). In addition, snpEff (Cingolani et al. 2012) was used to estimate the effect of polymorphisms on coding sequences. Data for *Vrs1* and *Int-c* were retrieved by inspecting the corresponding Morex WGS contigs (contig_135757, *Vrs1*, and contig_5747, *Int-c*), as identified by BLASTN alignment at http://webblast.ipk-gatersleben.de/barley_ibsc/viroblast.php.

RNA extraction and reverse-transcription PCR

Total RNA was extracted from immature spikes, leaf blades, leaf sheaths, nodes and internodes at awn primordium stage using TRIzol (Invitrogen). RNA was quantified using a NanoDrop 2000 (Thermo Fisher Scientific). To remove genomic DNA contamination, RNA was treated with RNase-free DNase I (Roche). First-strand cDNA was synthesized with SuperScript III (Invitrogen) and first-strand cDNA derived from 20 ng RNA was used as PCR template. Primers used for RT-PCR are listed in Supplementary Table 2. Barley *Actin* gene was used as positive control.

Phylogenetic analysis

Over two hundred *Vrs1* nucleotide sequences were retrieved with BLASTN from the NCBI nt database, using the sequence of SBCC153 (1,133 bp) as query. Hits with low similarity or query coverage, as well as redundant ones, were removed. The surviving sequences were trimmed to the length of SBCC153 and renamed using the allele and haplotype names defined in the work of Saisho et al. (2009). Haplotypes “hap13” and “hap3.2” were also filtered out for bearing large deletions. Sequences for barley genotypes widely used as standards for genomic studies, Barke, Bowman, Haruna Nijo and Morex *Vrs1* were included. A multiple alignment was computed with clustal-omega-1.2.1 (Sievers et al. 2011). A parsimony haplotype network was generated with software TCS v1.21 (Clement et al. 2000) and default parameters, which consider gaps as a fifth character. The resulting phylogenetic tree was optimally plotted with http://cibio.up.pt/software/tcsBU (dos Santos et al. 2016).

Results

SNPs genotypes of *Vrs1*

This study investigates 215 accessions that offer a good perspective of the diversity of the crop, with particular focus on the western Mediterranean region (Table 1). *Vrs1* alleles were initially inferred combining spike row-type and the four SNPs within *Vrs1* presented by the 9k Illumina Infinium assay (Cuesta-Marcos et al. 2010). The SNP markers provided information to discriminate among genotypes (Table 2). BOPA marker 12_30897 (G/A) differentiates *Vrs1.b3* (two-rowed) and *vrs1.a3* (six-rowed) from the other alleles. Marker 12_30900 (C/G) is specific to the *vrs1.a3* allele. Marker 12_30896 (G/A), although it does not contribute to function of *Vrs1*, can be used to identify six-rowed barleys with the *vrs1.a1* allele from the rest. The last marker 12_30901 (G/A) separates one of the major branches of the phylogenetic tree of *Vrs1*

(with alleles *Vrs1.b2*, *vrs1.a2* and *Vrs1.t*) from the other two major branches of cultivated barley (with alleles *vrs1.a1*, *Vrs1.b3* and *vrs1.a3*). The BOPA scores of the 215 accessions are shown in Supplementary Table 1.

Sequence validation of *Vrs1* alleles

Sanger sequencing of 28 accessions were also carried out (Supplementary Table 1). The sequence data discriminated between some non-committal genotypes and concluded all the allele calls by SNP genotyping. As a whole, nine polymorphisms differentiated seven *Vrs1* alleles (Table 2). The sequences of lines identified as *vrs1.a1*, *vrs1.a2*, *Vrs1.b2*, *Vrs1.b3*, and *Vrs1.t* were identical to the sequences downloaded from NCBI. A new allele, named *Vrs1.b5*, was found in seven two-rowed Spanish accessions. Exome sequencing of 73 genotypes (mostly Spanish landraces) independently confirmed the polymorphisms identified (Supplementary Table 3), providing full allelic discrimination. Thus, 26 lines were correctly classified as *vrs1.a1*, 37 lines were *vrs1.a2*, 2 lines had the *vrs1.a3* allele, and 7 carried the *Vrs1.b3* allele and 1 as *Vrs1.b5*.

Discovery of a novel allele *Vrs1.b5*

The new *Vrs1* allele was identified in seven two-rowed Spanish landraces (Fig. 1A). This allele has been named *Vrs1.b5*, the number being the next in sequence available for this gene. The *Vrs1.b5* allele, presents the distinctive thymine ('T') insertion in exon 2, typical of *vrs1.a2* but, on top of it, there was a single base deletion G/_ in the same exon, 45 bp upstream of this insertion. The coupled deletion/insertion results in a frameshift of a stretch of 15 amino acids (Fig. 1B) and restored two-rowed spike from the six-rowed spike (Fig. 1C). The frameshift was located outside of the homeodomain, in a region apparently not relevant for the function of the DNA-binding domain (Fig. 1B). The spike phenotype of the lines carrying the new allele is definitely two-rowed (Fig. 1C), indicating that the 15 substituted amino acids were not essential for the function of VRS1 in terms of suppressing the development of lateral florets. This change, a single base deletion, has not been observed in any other sequence reported for this gene. A molecular phylogeny analysis with 47 unique sequences from both domesticated and wild barleys, positioned the new *Vrs1.b5* allele only one-step apart from the six-row *vrs1.a2* allele, both sharing *Vrs1.b2* as common ancestor (Fig. 2).

196

197 **Expression of *Vrs1.b5***

198 Transcript of *Vrs1* was detected in the two accessions (SBCC153 and SBCC155) carrying *Vrs1.b5*
199 (Fig. 3). *Vrs1* was predominantly expressed in the immature spikes, as previously reported
200 (Sakuma et al. 2010, 2013). A six-rowed accession (SBCC039) carrying *vrs1.a2*, the immediate
201 ancestor of *Vrs1.b5* allele, showed the same gene expression pattern with the *Vrs1.b5* carriers.
202 Implication was that the gain of *Vrs1* function in *Vrs1.b5* was effected by the frameshift, not by
203 any change of transcription.

204

205 **Diversity and geographical distribution of *Vrs1* alleles**

206 Using the four SNPs from Cuesta-Marcos et al. 2010, together with the morphological
207 identification of the row number, allows the precise identification of most, but not all, *Vrs1*
208 alleles. To illustrate this, we predicted the *Vrs1* allele for another study involving 138 European
209 winter cultivars (Digel et al. 2016, Supplementary Table 4). Marker-based assignment of *Vrs1*
210 alleles allowed unequivocal identification of 60 *Vrs1.b3* alleles, 4 *vrs1.a1*, and 30 *vrs1.a3*. For
211 the rest, marker information complemented with spike row-type allowed assigning allele
212 *vrs1.a2* to 39 cultivars, and five cultivars (all two-rowed) were still inconclusive. Scoring the
213 rest of polymorphisms in Table 2 would allow further differentiation of the *Vrs1.b2*, *Vrs1.b5*
214 and *Vrs1.*t alleles.

215 We could also predict correctly row type and specific allele for 109 of the 126 landraces
216 studied by Russell et al. (2016). We retrieved the data for SNPs and indels within *Vrs1* (Morex
217 WGS contig_135757) from that study, identifying four polymorphic sites of the nine presented
218 in Table 2 (Supplementary Table 5). Most of the 72 six-rowed accessions carried the *vrs1.a1*
219 allele (52), originating from Asia and in Africa; *vrs1.a3* was present in 13 accessions, mainly
220 from Eastern Europe, and *vrs1.a2* was only found in 4 landraces from Spain and the French
221 Pyrenees. Regarding two-rowed accessions, 39 were identified as having the *Vrs1.b3* allele.
222 Further 13 accessions were identified as carrying one of the *Vrs1.b2* alleles, and four could not
223 be determined due to missing data.

224

225 **Association of *Vrs1* and *EF-G* alleles**

Previously, variation in the *EF-G* locus, closely linked to *Vrs1*, was used to infer different origins within cultivated barley (Tanno et al. 1999, 2002; Casas et al. 2005), and allows further differentiating the *Vrs1.t* allele (A-type) from the *Vrs1.b2* and *vrs1.a2* lineage (D-type). Most two-rowed accessions analyzed in this study (57 out of 72) had the *Vrs1.b3* allele, associated with the K-type in *EF-G*. Twelve two-rowed lines, (3 wild, 2 landraces from Morocco and 7 Spanish landraces), however, showed a D-type, which is typical of six-rowed lines.

The wild barleys and cultivated landraces from Morocco all carried the *Vrs1.b2* allele, as expected, as a similar finding was reported previously by Komatsuda et al. (2007).

Among 143 six-rowed accessions analyzed, 83 carried *vrs1.a2* allele, and 78 of the *vrs1.a2* accessions carried the D-type of EF-G (*cMWG699*) confirming their tight linkage (Komatsuda et al. 1999) and association (Tanno et al. 2002). The *vrs1.a2* was derived from *Vrs1.b2* (Fig. 3) as described earlier (Komatsuda et al. 2007) and all the *Vrs1.b2* carriers were D-type carriers (Table 2, Supplementary Table 1).

All the accessions carrying *Vrs1.b5* and most of the six-rowed accessions (78 out of 83) with *vrs1.a2* also carried the D-type at the *EF-G* locus, indicating that both alleles belong to the same lineage, an implication of the restoration of gene function in *Vrs1.b5* from *vrs1.a2* due to the deletion. The *Int-c* gene that intervenes in the size of the lateral spikelets was sequenced in several lines (Supplementary Table 1). Using Sanger and exome sequence data available for 75 accessions, six-rowed lines carried the *Int-c.a* allele, as expected, whereas seven two-rowed lines, including two with the *Vrs1.b5* allele, were *int-c.b1*, typical of two-rowed cultivated lines (Ramsay et al. 2011).

Discussion

The present study adds a new two-rowed allele *Vrs1.b5* to the catalogue of *Vrs1* diversity. The new allele was created by a restoration of gene function in the ancestral recessive allele by a single nucleotide deletion. This sort of mutation, a kind of gain-of function, is unusual in nature. The direction of mutation from recessive *vrs1* to dominant *Vrs1* was opposite to the normal direction so far discovered (Komatsuda et al. 2007). In the present study, we identified 7 two-rowed lines with the *Vrs1.b5* allele, out of 50 two-rowed Spanish lines analyzed, i.e., 14% of two-rowed Spanish barleys carry the new allele. The phenotype of these lines is definitely two-rowed, and two accessions surveyed for *int.c* carry the allele typically found in

two-rowed lines (Ramsay et al. 2011). A maximum parsimony phylogenetic analysis suggests that *Vrs1.b5* was a reversion of *vrs1.a2* to the two-rowed state through a new mutation. The reversion of the six-rowed to two-rowed seems far more uncommon than the opposite in the history of the crop (Komatsuda et al. 2007). Phylogenetically, loss-of-function allele *vrs1.a2* seems to derive from *Vrs1.b2*. Later, a deletion in *vrs1.a2* likely gave rise to *Vrs1.b5*, which restored the ORF and reverted to the two-rowed phenotype. Thirulogachandar et al. (2017) performed a phylogenetic analysis for plants HD-ZIP I proteins, identifying putative motifs evolutionary conserved. Motif 16, 14 amino acids long starting from Lys29, which corresponds to part of the 15 amino acid region changed in the *Vrs1.b5* allele, separated the monocot from the dicot proteins, as reported by those authors. The amino acid change in *Vrs1.b5* allele did not affect functionality of the protein although the motif was predicted to have a nuclear localization signal ('RRRRRRSAR').

Origin of *Vrs1.b5*

The presence of the *Vrs1.b2* to *vrs1.a2* lineage in the western Mediterranean was previously reported by Komatsuda et al. (2007), who proposed that the *vrs1.a2* allele could be native to the region. This view was supported by studies carried out with the *EF-G* locus (marker *cMWG699*). Several surveys done with this marker concluded that the D-type (associated to *vrs1.a2* and *Vrs1.b2*) was found preferentially in the Mediterranean region (Tanno et al. 1999, 2002; Casas et al. 2005; Baba et al. 2011), but it was also present in winter six-rowed cultivars from Germany, France, and other western European countries (Casas et al., 2005). Since both two- and six-rowed barleys carrying the D allele are present in North Africa, Baba et al. (2011) proposed that the origin of the D allele was in Morocco. Our results illustrate that the D allele is profusely present in Spanish six-rowed landraces, most of them with the *vrs1.a2* allele, and in a small group of two-rowed landraces featuring the new *Vrs1.b5* allele. Moreover, even today, a large proportion of six-rowed *vrs1.a2* genotypes can still be found among European modern winter cultivars, as derived from data provided in Digel et al. (2016). Therefore, its geographic origin cannot be indicated with certainty. *Vrs1.b2* probably appeared in the Middle East. Recent sequencing of a 6,000-year-old barley from a cave in Israel revealed that it carried a putative two-rowed *Vrs1.b2* genotype (Mascher et al. 2016). *Vrs1.b2* has also been found in the old landrace Palmella Blue (Komatsuda et al. 2007), collected in Egypt early in the 20th century (https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?id=1025310). In this study, we have found this allele in two landraces from Morocco which, could represent

290 remains of the same genetic stock, found at the two ends of its geographical distribution after
291 westwards expansion though the Mediterranean during the Neolithic (Zilhao 2011).

292 Considering together the presence of the precursor allele of *Vrs1.b5* (*vrs1.a2*) exclusively in
293 Western Europe and, in lower frequencies, in Morocco (Casas et al. 2005, Baba et al. 2011, and
294 data derived from Digel et al. 2016, and Russell et al. 2016), and the discovery of *Vrs1.b5* in
295 Spanish landraces, we hypothesize that this new allele is native to the western part of the Old
296 World.

297

298 **Application of the SNP markers for germplasm characterization**

299 An updated classification of the *Vrs1* alleles can facilitate the analysis and differentiation of
300 genotypes. Earlier efforts by Cockram et al. (2012), who developed a set of KASP markers for
301 morphological traits assessed to determine distinctiveness, uniformity and stability (DUS) of
302 new plant breeding varieties, including *Vrs1*, did not differentiate among all possible alleles.
303 Similarly, the four SNP markers developed by Cuesta-Marcos et al. (2010) are not fully
304 diagnostic, but at least allow an easy first discriminating step that could be implemented in all
305 datasets based on the 9K and 50K (Bayer et al. 2017) barley chips. We illustrated its application
306 by correctly estimating the *Vrs1* allele for 133 of the 138 cultivars evaluated by Digel et al.
307 (2016), with five inconclusive (Supplementary Table 4). Four of the remaining genotypes
308 shared a common parent with the *Vrs1.t* allele (Intro) in their pedigree, and two of them have
309 recently been identified as *deficiens* (Sakuma et al. 2017).

310 Different studies carried out genome wide association analysis for type of spike or yield related
311 traits and found QTL in the region of the *Vrs1* locus. Even though Cuesta-Marcos et al. (2010)
312 developed the 4 SNP BOPA markers described in Table 2, these authors reported more highly
313 significant associations by the use of 'synthetic markers' which summarized the
314 dominant/recessive nature of the *Vrs1* allele than with any SNP within the gene. In another
315 genome-wide study with six-rowed cultivars and advanced breeding lines, Berger et al. (2013)
316 identified markers in the *vrs1* region associated with QTL for test weight but were not able to
317 differentiate the lines. The authors concluded that either there was a closely linked gene for
318 test weight, or there were two or more *vrs1* alleles segregating in the breeding materials with
319 one of them contributing directly to increased test weight. An examination of the specific *Vrs1*
320 alleles present in their dataset would have shed further light on these hypotheses. In the work

by Muñoz-Amatriaín et al. (2014), with the USDA barley core collection, the largest panel tested up to date, the top hit for spike row number corresponded to marker 12_30896. This marker separates six-rowed *vrs1.a1* accessions from the rest, which includes not only 2-rowed accessions, but also other 6-rowed ones with different alleles. Therefore, this marker does not discriminate row type. It was captured by GWAS probably because there was an imbalance of allelic frequencies in the genotypes, with a majority of 6-rowed presenting *vrs1.a1*. In the last study published by Thirulogachandar et al. (2017), the two SNPs associated with variation in leaf area are 12_30896 and 12_30900 (both within *Vrs1* and differentiating six-rowed lines with allele *vrs1.a1* or *vrs1.a3*, respectively). Overall, a comprehensive evaluation of *Vrs1* for the markers differentiating all alleles, as haplotypes instead of considering them independently, would allow an accurate characterization of the alleles present in barley materials as the ones reported in those studies. A definitive allele characterization could provide new insights on association of specific *Vrs1* alleles to the relevant agronomic and morphological traits reportedly related to this gene.

This study completes the allelic catalogue of gene *Vrs1*, offers new insights on explanations for their geographic distribution, and provides a full list of SNP markers useful for breeders and germplasm banks to better analyse genetic variation associated to this gene, and facilitate germplasm classification.

Data accessibility

Newly reported sequences for *Int-c* and *Vrs1* are accessible at European Nucleotide Archive under references LT727691-LT727723.

Authors' contributions

AMC, EI and TK conceived this work. PG, MM and JMC selected and provided the plant accessions. AMC and SS performed laboratory work. AMC, CPC and BCM analyzed the DNA sequence data. BCM was responsible for the phylogenetic analysis. AMC, BCM, EI and TK drafted the document. All the authors read and approved the manuscript.

Acknowledgments

The authors would like to thank Dr. Alessandro Tondelli, who provided data for marker 12_30896 on the barley genotypes tested by Digel et al. (2016). This work was supported by

Spanish Ministry of Economy, Industry and Competitiveness grants AGL2010-21929, AGL2013-48756-R, RFP2012-00015-00-00, RTA2012-00033-C03-02 and EUI2009-04075 (national code for Plant-KBBE project ExpResBar). CPC was funded by Spanish Ministry of Economy, Industry and Competitiveness grant no. BES-2011-045905 (linked to project AGL2010-21929). TK and SS were supported by a research fund by the Ministry of Agriculture, Forestry, and Fisheries of Japan (Genomics for Agricultural Innovation grants no. TRS1002). SS was supported by a Grant-in-Aid from the Japan Society for the Promotion of Science (JSPS) Postdoctoral Fellow for Research Abroad and a Grant-in-Aid for Young Scientists (B) (no. 16K18635).

Reference List

Alqudah AM, Koppolu R, Wolde GM, Graner A, Schnurbusch T (2016) The genetic architecture of barley plant stature. Front Genet 7:117. doi: 10.3389/fgene.2016.00117

Baba T, Tanno K, Furusho M, Komatsuda T (2011) Allelic variation at the *EF-G* locus among northern Moroccan six-rowed barleys. Plant Genet Resour: Charact Util 9:240–242. doi: 10.1017/S1479262111000451

Banks WE, Antunes N, Rigaud S, d'Errico F (2013) Ecological constraints on the first prehistoric farmers in Europe. Journal of Archaeological Science 40:2746-2753. doi: 10.1016/j.jas.2013.02.013

Bayer MM, Rapazote-Flores P, Ganal M, Hedley PE, Macaulay M, Plieske J, Ramsay L, Russell J, Shaw PD, Thomas W, Waugh R (2017) Development and evaluation of a barley 50k iSelect SNP array. Front Plant Sci 8:1792. doi: 10.3389/fpls.2017.01792

Berger GL, Liu S, Hall MD, Brooks WS, Chao S, Muehlbauer GJ, Baik BK, Steffenson B, Griffey CA (2013) Marker-trait associations in Virginia Tech winter barley identified using genome-wide mapping. Theor Appl Genet 126: 693–710. doi: 10.1007/s00122-012-2011-7

Bull H, Casao MC, Zwirek M, Flavell AJ, Thomas WTB, Guo W, Zhang R, Rapazote-Flores P, Kyriakidis S, Russell J, Druka A, McKim SM, Waugh R (2017) Barley SIX-ROWED SPIKE3 encodes a putative Jumonji C-type H3K9me2/me3 demethylase that represses lateral spikelet fertility. Nat Comm 8: 936. doi: 10.1038/s41467-017-00940-7

Cantalapiedra CP, Contreras-Moreira B, Silvar C, Perovic D, Ordon F, Gracia MP, Igartua E, Casas AM (2016) A cluster of Nucleotide-Binding Site–Leucine-Rich Repeat genes resides in a barley powdery mildew resistance Quantitative Trait Loci on 7HL. Plant Genome 9:2 doi: 10.3835/plantgenome2015.10.0101

382 Casas AM, Yahiaoui S, Ciudad F, Igartua E (2005) Distribution of MWG699 polymorphism in
383 Spanish European barleys. Genome 48:41–45. doi: 10.1139/G04-091

384 Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM (2012) A
385 program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff.
386 Fly 6:80-92. doi: 10.4161/fly.19695

387 Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene
388 genealogies. Mol. Ecol 9:1657-1659. doi: 10.1046/j.1365-294x.2000.01020.x

389 Cockram J, Jones H, Norris C, O'Sullivan DM (2012) Evaluation of diagnostic molecular markers
390 for DUS phenotypic assessment in the cereal crop, barley (*Hordeum vulgare* ssp. *vulgare* L.).
391 Theor Appl Genet 125:1735–1749. doi: 10.1007/s00122-012-1950-3

392 Comadran J, Kilian B, Russell J, Ramsay L, Stein N, Ganal M, Shaw P, Bayer M, Thomas W,
393 Marshall D, Hedley P, Tondelli A, Pecchioni N, Francia E, Korzun V, Walther A, Waugh R (2012)
394 Natural variation in a homolog of *Antirrhinum CENTRORADIALIS* contributed to spring growth
395 habit and environmental adaptation in cultivated barley. Nat Genet 44:1388-1392. doi:
396 10.1038/ng.2447

397 Cuesta-Marcos A, Szűcs P, Close TJ, Filichkin T, Muehlbauer GJ, Smith KP, Hayes PM (2010)
398 Genome-wide SNPs and re-sequencing of growth habit and inflorescence genes in barley:
399 implications for association mapping in germplasm arrays varying in size and structure. BMC
400 Genomics 11:707. doi: 10.1186/1471-2164-11-707

401 Digel B, Tavakol E, Verderio G, Tondelli A, Xu X, Cattivelli L, Rossini L, von Korff M (2016)
402 Photoperiod-H1 (Ppd-H1) locus controls leaf size. Plant Physiol 172:405-415. doi:
403 10.1104/pp.16.00977

404 dos Santos AM, Cabezas MP, Tavares AI, Xavier R, Branco M (2016) tcsBU: a tool to extend TCS
405 network layout and visualization. Bioinformatics 32:627-628. doi:
406 10.1093/bioinformatics/btv636

407 Evans LT, IF Wardlaw (1976) Aspects of the comparative physiology of grain yield in cereals.
408 Adv Agron 28: 301–359. doi: 10.1016/S0065-2113(08)60558-1

409 Fischbeck G (2003). Diversification through breeding. In: von Bothmer R, van Hintum Th,
410 Knüpffer H, Sato K (eds) Diversity in Barley (Hordeum vulgare). Elsevier Science BV,
411 Amsterdam, The Netherlands. pp 29-52

412 Igartua E, Gracia MP, Lasa JM, Medina B, Molina-Cano JL, Montoya JL, Romagosa I (1998) The
413 Spanish barley core collection. Genet Resour Crop Evol 45:475-481. doi:
414 10.1023/A:1008662515059

415 Komatsuda T, Nakamura I, Takaiwa F, Oka S (1998) Development of STS markers closely linked
416 to the *vrs1* locus in barley, *Hordeum vulgare*. Genome 41:680–685. doi: 10.1139/g98-069

417 Komatsuda T, Li W, Takaiwa F, Oka S (1999) High resolution map around the *vrs1* locus
418 controlling two- and six-rowed spike in barley, *Hordeum vulgare*. Genome 42:248–2535.
419 https://doi.org/10.1139/g98-132

420 Komatsuda T, Pourkheirandish M, He C, Azhaguvel P, Kanamori H, Perovic D, Stein N, Graner A,
421 Wicker T, Tagiri A, Lundqvist U, Fujimura T, Matsuoka M, Matsumoto T, Yano M (2007) Six-
422 rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox
423 gene. Proc Natl Acad Sci USA 104:1424–1429. doi: 10.1073/pnas.0608580104

424 Koppolu R, Anwar N, Sakuma S, Tagiri A, Lundqvist U, Pourkheirandish M, Rutten T, Seiler C,
425 Himmelbach A, Ariyadasa R, Youssef HM, Stein N, Sreenivasulu N, Komatsuda T, Schnurbusch T
426 (2013) Six-rowed spike4 (Vrs4) controls spikelet determinacy and row-type in barley. Proc Natl
427 Acad Sci USA 110: 13198-13203. doi: 10.1073/pnas.1221950110

428 Leino MW, Hagenblad J (2010) Nineteenth century seeds reveal the population genetics of
429 landrace barley (*Hordeum vulgare*). Mol Biol Evol 27:964–973. doi: 10.1093/molbev/msp308

430 Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler Transform.
431 Bioinformatics 25:1754–1760. doi: 10.1093/bioinformatics/btp324

432 Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000
433 Genome Project Data Processing Subgroup (2009) The Sequence Alignment/Map format and
434 SAMtools. Bioinformatics 25:2078–2079. doi: 10.1093/bioinformatics/btp352

435 Liller CB, Neuhaus R, von Korff M, Koornneef M, van Esse W (2015) Mutations in barley row
436 type genes have pleiotropic effects on shoot branching. PLoS ONE 10: e0140246. doi:
437 10.1371/journal.pone.0140246

438 Lister DL, Jones H, Jones MK, O'Sullivan DM, Cockram J (2013) Analysis of DNA polymorphism
439 in ancient barley herbarium material: Validation of the KASP SNP genotyping platform. Taxon
440 62:779-789. doi: 10.12705/624.9

441 Mascher M, Richmond TA, Gerhardt DJ, Himmelbach A, Clissold L, Sampath D, Ayling S,
442 Steuernagel B, Pfeifer M, D'Ascenzo M, Akhunov ED, Hedley PE, Gonzales AM, Morrell PL,

Kilian B, Blattner FR, Scholz U, Mayer KFX, Flavell AJ, Muehlbauer GJ, Waugh R, Jeddeloh JA, Stein N (2013) Barley whole exome capture: a tool for genomic research in the genus *Hordeum* and beyond. Plant J 76:494-505. doi: 10.1111/tpj.12294

Mascher M, Schuenemann VJ, Davidovich U, Marom N, Himmelbach A, Hübner S, Korol A, David M, Reiter E, Riehl S, Schreiber M, Vohr SH, Green RE, Dawson IK, Russell J, Kilian B, Muehlbauer GJ, Waugh R, Fahima T, Krause J, Weiss E, Stein N (2016) Genomic analysis of 6,000-year-old cultivated grain illuminates the domestication history of barley. Nat Genet 48:1089-1093. doi: 10.1038/ng.3611

Mascher M, Gundlach H, Himmelbach A, Beier S, Twardziok SO, Wicker T, Radchuk V, Dockter C, Hedley PE, Russell J, Bayer M, Ramsay L, et al. (2017). A chromosome conformation capture ordered sequence of the barley genome. Nature 544:427-433. doi: 10.1038/nature22043

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA (2010) The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20:1297–1303. doi: 10.1101/gr.107524.110

Molina-Cano JL, Gómez-Campo C, Conde J (1982) *Hordeum spontaneum* C. Koch as a weed of barley fields in Morocco. Z Pflanzenzüchtg 88:161–167

Molina-Cano JL, Russell JR, Moralejo MA, Escacena JL, Arias G, Powell W (2005) Chloroplast DNA microsatellite analysis supports a polyphyletic origin for barley. Theor Appl Genet 110:613-619. doi: 10.1007/s00122-004-1878-3

Moralejo M, Romagosa I, Salcedo G, Sánchez-Monge R, Molina-Cano JL (1994) On the origin of Spanish two-rowed barleys. Theor Appl Genet 87:829-836. doi: 10.1007/BF00221135

Müller J (2015). Movements of plants, animals, ideas and people in South-East Europe. In: The Oxford Handbook of Neolithic Europe, pp 63-80. Edited by Chris Fowler, Jan Harding, and Daniela Hofmann. OUP (Oxford University Press)

Muñoz-Amatriaín M, Cuesta-Marcos A, Endelman JB, Comadran J, Bonman JM, Bockelman HE, Chao S, Russell J, Waugh R, Hayes PM, Muehlbauer GJ (2014) The USDA barley core collection: genetic diversity, population structure, and potential for Genome-Wide Association Studies. PLoS ONE 9:e94688. doi: 10.1371/journal.pone.0094688

Pujol-Andreu J (2011) Wheat varieties and technological change in Europe, 19th and 20th centuries: New issues in economic history. Historia Agraria 54: 71-103.

Ramsay L, Comadran J, Druka A, Marshall DF, Thomas WTB, Macaulay M, MacKenzie K, Simpson C, Fuller J, Bonar N, Hayes PM, Lundqvist U, Franckowiak JD, Close TJ, Muehlbauer GJ, Waugh R (2011) *INTERMEDIUM-C*, a modifier of lateral spikelet fertility in barley, is an ortholog of the maize domestication gene *TEOSINTE BRANCHED 1*. Nat Genet 43:169-172. doi: 10.1038/ng.745

Russell J, Mascher M, Dawson IK, Kyriakidis S, Calixto C, Freund F, Bayer M, Milne I, Marshall-Griffiths T, Heinen S, Hofstad A, Sharma R, Himmelbach A, Knauft M, van Zonneveld M, Brown JWS, Schmid K, Kilian B, Muehlbauer GJ, Stein N, Waugh R (2016) Exome sequencing of geographically diverse barley landraces and wild relatives gives insights into environmental adaptation. Nat Genet 48:1024-1030. doi: 10.1038/ng.3612

Saisho D, Pourkheirandish M, Kanamori H, Matsumoto T, Komatsuda T (2009) Allelic variation of row type gene *Vrs1* in barley and implication of the functional divergence. Breed Sci 59:621-628. doi: 10.1270/jsbbs.59.621

Sakuma S, Pourkheirandish M, Matsumoto T, Koba T, Komatsuda T (2010) Duplication of a well-conserved homeodomain-leucine zipper transcription factor gene in barley generates a copy with more specific functions. Funct Integr Genomics 10: 123–133. doi: 10.1007/s10142-009-0134-y

Sakuma S, Pourkheirandish M, Hensel G, Kumlehn J, Stein N, Tagiri A, Yamaji N, Ma JF, Sassa H, Koba T, Komatsuda T (2013) Divergence of expression pattern contributed to neofunctionalization of duplicated HD-Zip I transcription factor in barley. New Phytol 197:939-948. doi: 10.1111/nph.12068

Sakuma S, Lundqvist U, Kakei Y, Thirulogachandar V, Suzuki T, Hori K, Wu J, Tagiri A, Rutten T, Koppolu R, Shimada Y, Houston K, Thomas WTB, Waugh R, Schnurbusch T, Komatsuda T (2017) Extreme suppression of lateral floret development by a single amino acid change in the VRS1 transcription factor. Plant Physiol 175:1720-1731. doi: 10.1104/pp.17.01149

Sievers F, Wilm A, Dineen DG, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins DG (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 7:539. doi: 10.1038/msb.2011.75

Tanno K, Takaiwa F, Oka S, Komatsuda T (1999) A nucleotide sequence linked to the *vrs1* locus for studies of differentiation in cultivated barley (*Hordeum vulgare* L.). Hereditas 130:77-82. doi: 10.1111/j.1601-5223.1999.00077.x

506 Tanno K, Taketa S, Takeda K, Komatsuda T (2002) A DNA marker closely linked to the *vrs1* locus
507 (row-type gene) indicates multiple origins of six-rowed cultivated barley (*Hordeum vulgare* L.).
508 Theor Appl Genet 104:54-60. doi: 10.1007/s001220200006

509 Thirulogachandar V, Alqudah AM, Koppolu R, Rutten T, Graner A, Hensel G, Kumlehn J,
510 Bräutigam A, Sreenivasulu N, Schnurbusch T, Kuhlmann M (2017) Leaf primordium size
511 specifies leaf width and vein number among row-type classes in barley. Plant J 91: 601-612.
512 doi: 10.1111/tpj.13590

513 van Esse GW, Walla A, Finke A, Koornneef M, Pecinka A, von Korff M (2017) Six-rowed spike3
514 (VRS3) is a histone demethylase that controls lateral spikelet development in barley. Plant
515 Physiol 174: 2397-2408. doi/10.1104/pp.17.00108

516 von Bothmer R, Sato K, Komatsuda T, Yasuda Y, Fischbeck G (2003). The domestication of
517 cultivated barley. In: von Bothmer R, van Hintum Th, Knüpffer H, Sato K (eds) Diversity in
518 Barley (Hordeum vulgare). Elsevier Science BV, Amsterdam, The Netherlands. pp 9-27

519 Youssef HM, Koppolu R, Schnurbusch T (2012) Re-sequencing of *vrs1* and *int-c* loci shows that
520 *labile* barleys (*Hordeum vulgare* convar. *labile*) have a six-rowed genetic background. Genet
521 Resour Crop Evol 59:1319–1328. doi: 10.1007/s10722-011-9759-5

522 Youssef HM, Eggert K, Koppolu R, Alqudah AM, Poursarebani N, Fazeli A, Sakuma S, Tagiri A,
523 Rutten T, Govind G, Lundqvist U, Graner A, Komatsuda T, Sreenivasulu N, Schnurbusch T
524 (2017a) VRS2 regulates hormone-mediated inflorescence patterning in barley. Nat Genet 49:
525 157-161. doi:10.1038/ng.3717

526 Youssef HM, Mascher M, Ayoub MA, Stein N, Kilian B, Schnurbusch T (2017b) Natural diversity
527 of inflorescence architecture traces cryptic domestication genes in barley (*Hordeum vulgare*
528 L.). Genet Resour Crop Evol 64:843-853. doi: 10.1007/s10722-017-0504-6

529 Zilhao J (2000) From the Mesolithic to the Neolithic in the Iberian Peninsula. In Price TD (ed),
530 Europe's First Farmers, Cambridge University Press, Cambridge, pp. 144–182.

531

532 **Table 1** Plant materials and genotyping platforms used in this study.

Country/ Region	Type of material	Spike row-type	No.	Genotyping	Reference
Morocco	Wild	Two-rowed	3	Eureka Genomics, EF-G, Sanger	Molina-Cano et al. 1982
Morocco	Landraces	Two- and six-rowed	8	Eureka Genomics, EF-G, Sanger (3)	Moralejo et al. 1994, Molina Cano et al. 2005
Spain	Landraces	Two-rowed	39	Eureka Genomics, EF-G, Sanger (5)	Moralejo et al. 1994
Spain	Landraces	Two- and six-rowed	137	9k iSelect, EF-G, exome capture (66), Sanger (14)	Igartua et al. 1998
Europe/ USA	Cultivars	Two- and six-rowed	28	9k iSelect, EF-G, exome capture (7), Sanger (3)	This study

533

Table 2 *Vrs1* alleles detected in this study.

	Nucleotide positions referred to Morex WGS contig_135757 (*vrs1.a1* allele)											
	Exon 1	Exon 2				Intron 2	Exon 3		3' UTR	Row	EF-G	No. of
Allele	1067	1240	1246	1288	1393	1608	1725	1818	1961	type	type	accessions
Vrs1.b2	G	G	G	–	C	T	G	A	C	2	D	5
vrs1.a2	G	G	G	T	C	T	G	A	C	6	D	83
Vrs1.b5	G	–	G	T	C	T	G	A	C	2	D	7
vrs1.a1	G	G	G	–	C	C	–	A	T	6	A	57
Vrs1.b3	A	G	T	–	C	C	G	A	C	2	K	57
vrs1.a3	A	G	T	–	G	C	G	A	C	6	A	3
Vrs1.t1	G	G	G	–	C	T	G	G	C	2	A	3
Effect	Gly8>Asp	Glu26>F.S.	Glu26>Asp	Ala40>F.S.	Phe75>Leu		Glu152>F.S.	Ser184>Gly				
BOPA	12_30897				12_30900	12_30901			12_30896			

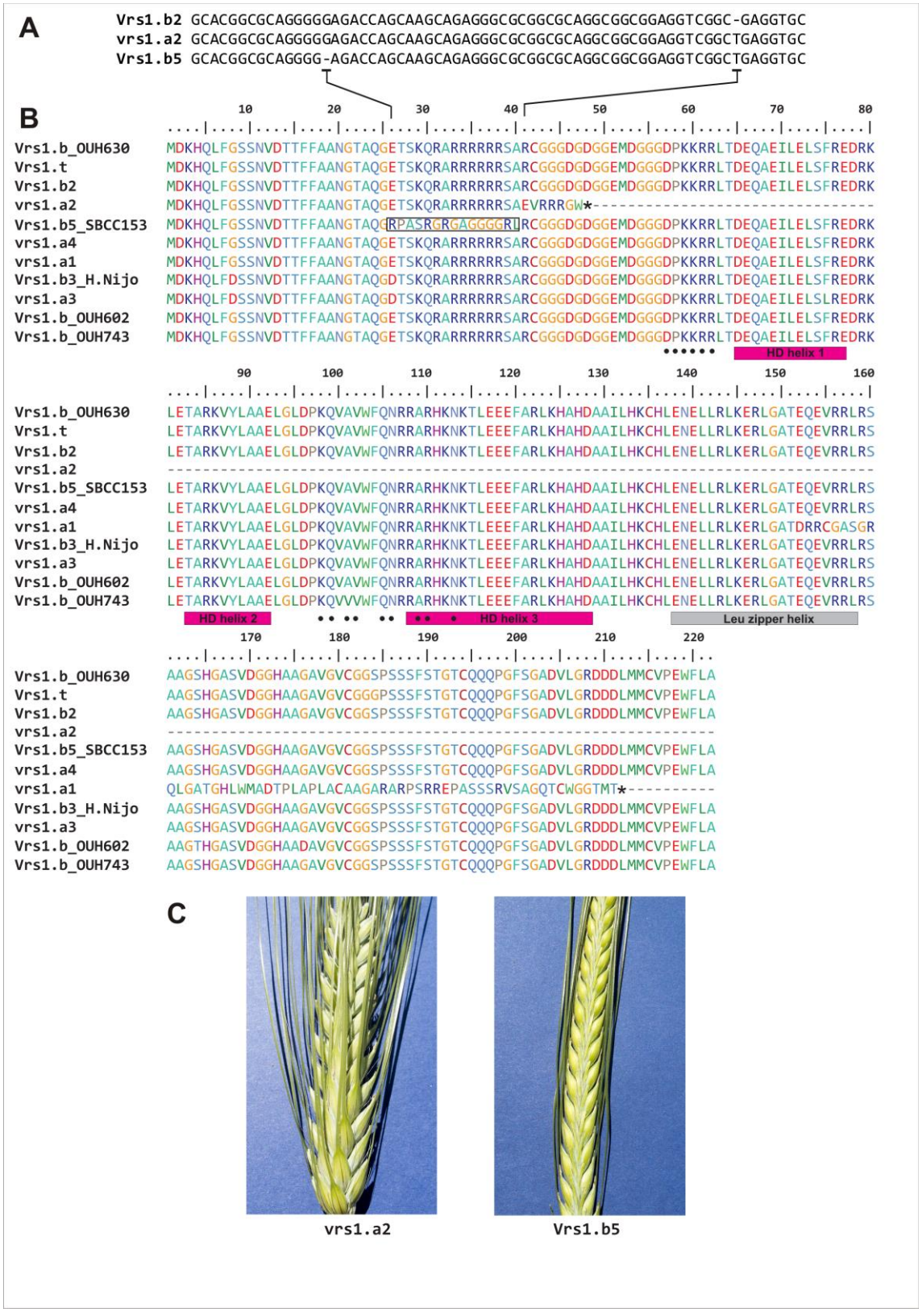
BOPA markers 12_30901 and 12_30896 interrogate the complementary strand, therefore are usually reported as A/G and G/A, respectively

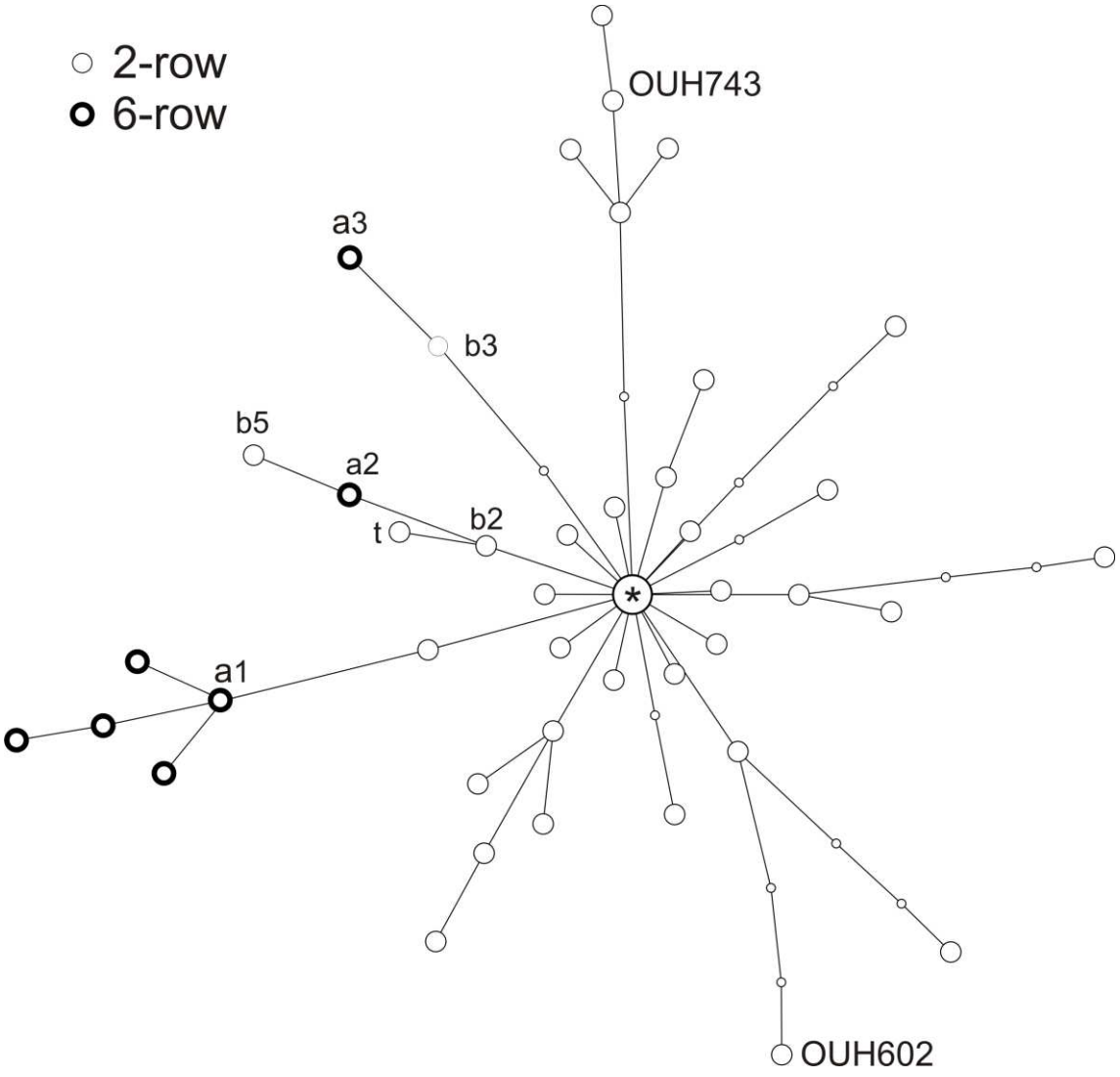
537 **Figure legends**

538 **Fig. 1** Discovery of *Vrs1.b5*. (A) Alignment of *Vrs1.b2*, *vrs1.a2* and *Vrs1.b5* DNA partial
539 sequences of exon 2 indicating the creation of *Vrs1.b5* by 1-bp deletion in *vrs1.a2*. (B) Multiple
540 alignment of protein sequences of different alleles of the *Vrs1* gene. Secondary structure
541 elements of the homeodomain (HD) and the leucine zipper dimerization domain (Leu zipper)
542 are shown as color-filled boxes. Predicted protein-DNA interfaces residues are marked with
543 circles. Boxed sequences highlight differences among alleles and asterisks mark premature
544 stop codons. Three wild barley lines (''OUH'' identifiers) used as outgroups in a previous
545 phylogeny (Komatsuda et al. 2007) are also shown. Alignment was computed with clustal-
546 omega-1.2.1. (C) Barley spikes from Spanish landraces SBCC039 (*vrs1.a2*) six-rowed (left) and
547 SBCC155 (*Vrs1.b5*) two-rowed (right).
548

549 **Fig. 2** Sequence analysis of 47 aligned *Vrs1* alleles. Parsimony network where edges represent
550 single-mutation transitions, and nodes correspond to haplotypes. Tiny circles represent
551 intermediate states (with no associated genotypes). Six-rowed barley accessions are displayed
552 as thick circles. The central haplotype corresponds to OUH630. The labeled circles match the
553 haplotypes shown in Figure 1B.
554

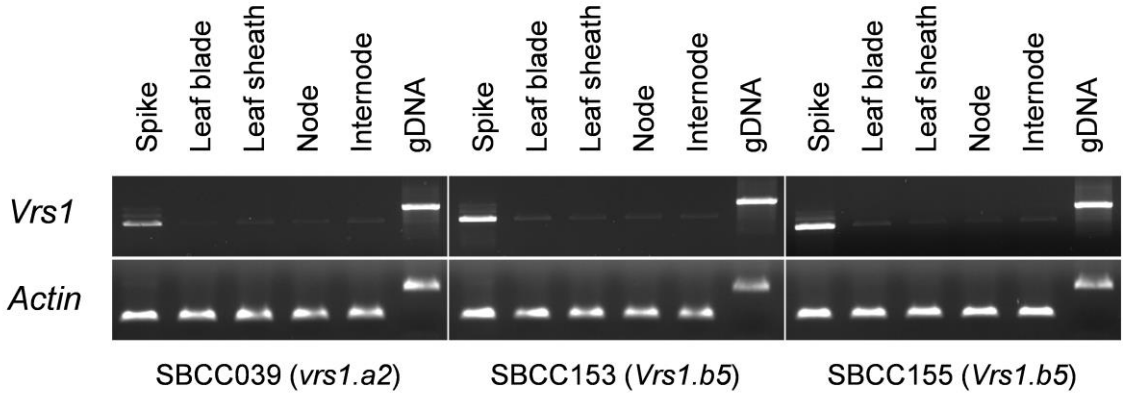
555 **Fig. 3** Reverse transcription PCR (RT-PCR) analysis of *Vrs1*
556 *Vrs1* was predominantly expressed in the immature spikes in both six-rowed (SBCC039) and
557 two-rowed barley (SBCC153 and SBCC155). All organs were collected from main tiller at the
558 awn primordium stage. The PCR product of *Vrs1* was directly sequenced to confirm the
559 specificity. *Actin* was used as a control.
560
561


A
Vrs1.b2 GCACGGCGCAGGGGGAGACCAGCAAGCAGAGGGCGCGGCGCAGGCGGCGGAGGTCGGC-GAGGTGC
vrs1.a2 GCACGGCGCAGGGGGAGACCAGCAAGCAGAGGGCGCGGCGCAGGCGGCGGAGGTCGGCTGAGGTGC
Vrs1.b5 GCACGGCGCAGGGG-AGACCAGCAAGCAGAGGGCGCGGCGCAGGCGGCGGAGGTCGGCTGAGGTGC
B
Vrs1.b_OUH630
Vrs1.t
Vrs1.b2
vrs1.a2
Vrs1.b5_SBCC153
vrs1.a4
vrs1.a1
Vrs1.b3_H.Nijo
vrs1.a3
Vrs1.b_OUH602
Vrs1.b_OUH743
HD helix 1
HD helix 2
HD helix 3
Leu zipper helix
C
vrs1.a2
Vrs1.b5



564

565


Spike
Leaf blade
Leaf sheath
Node
Internode
gDNA
Vrs1
Actin
SBCC039 (vrs1.a2)
SBCC153 (Vrs1.b5)
SBCC155 (Vrs1.b5)

566